AMENDMENTS TO THE SPECIFICATION

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Please replace the first paragraph on page 1 with the following amended paragraph:

--This is a continuation of U.S. Patent Application Serial No. 10/260,031, filed on June 6, 2003, which is a continuation of U.S. patent Application Serial No. ~~09.260,031~~ 09/302,817, filed on ~~April 16, 1999~~February 3, 1998, now abandoned. Serial No. 09/~~260,031~~09/302,817 is a divisional of Serial No. 08/182,621, filed on January 13, 1994, also abandoned.--

Please amend the "BRIEF DESCRIPTION OF THE DRAWINGS" on pages 10-12 as follows:

--BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 (A-F) depicts various nucleic acid construct forms contemplated by the invention in which at least one single-stranded region are located therein.

FIG. 2 (A-F) depicts the functional forms of the nucleic acid constructs illustrated in FIG. 1 (A-F).

FIG. 3 (A-C) is an illustration of three nucleic acid constructs with an RNA polymerase covalently attached to a transcribing cassette.

FIG. 4 (A-C) illustrates three nucleic acid constructs with promoters for endogenous RNA polymerase.

FIG. 5 is a nucleic acid sequence for M13mp18 (SEQ ID NO:1).

FIG. 6 shows the sequence and the positions of the primers 1-20 depicted in SEQ ID NOS:2-21 respectively derived from M13mp18 which were employed in the present invention for nucleic acid production.

FIG. 7 illustrates appropriate restriction sites in M13mp18.

FIG. 8 is an agarose gel with a lane legend illustrating the experimental results in Example 5 in which amplification of the M13 fragment was carried out in the presence of a large excess (1500 fold) of irrelevant DNA.

FIG. 9 is an agarose gel with a lane legend illustrating the results in Example 8 in which the effect of variations of reaction conditions on the product obtained in Example 3 was investigated.

FIG. 10 is an agarose gel with a lane legend that illustrates the results of a qualitative analysis of the effects observed in Example 9 of various buffers on the amplification reaction in accordance with the present invention.

FIG. 11 is a southern blot (with lane legend) obtained from Example 10 in which two buffers, DMAB and DMG, were separately employed in nucleic acid production.

FIG. 12 is an agarose gel and lane legend obtained in Example 11 in which the nature of the ends of amplified product was investigated.

FIG. 13 is an agarose gel obtained in Example 12 in which amplification from non-denatured template was examined.

FIG. 14 is an agarose gel obtained in Example 13 in which amplification from an RNA template was examined.

FIG. 15 is a southern blot of the gel obtained in FIG. 14.

FIG. 16 is a fluorescence spectrum illustrating the results obtained in Example 14 in which the phenomenon of "strand displacement" using ethidium-labeled oligonucleotides in accordance with the present invention was investigated.

FIG. 17 is a fluorescence spectrum illustrating the results obtained in Example 15 in which a T7 promoter oligonucleotide 50 mer labeled with ethidium was employed to study its effects on in vitro transcription by T7 and T3 polymerases from an IBI 31 plasmid (plbl 31-BH5-2) and from a BlueScript II plasmid construct (pBSII//HCV).

FIG. 18 depicts the polylinker sequences of the IBI 31 plasmid (plbl 31-BH5-2) (SEQ ID NOS:22-24)and the BlueScript II plasmid construct (pBSII//HCV) (SEQ ID NOS:25-27).

Please amend the paragraph bridging pages 14 and 15 as follows:
In addition, the specific nucleic acid can be in solution in which case the above-described *in vitro* process may further comprise the step of treating the specific nucleic acid with a blunt-end promoting restriction enzyme. Further, isolation or purification procedures can be employed to enrich the specific nucleic acid. Such procedures are well-known in the art, and may be carried out on the specific nucleic acid prior to the contacting step (b) or the reacting step (c). One means of isolation or purification of a nucleic acid involves its immobilization, for example, by sandwich hybridization (Ranki et al., 1983), or sandwich capture. Particularly significant in the latter methodology is the disclosure of Engelhardt and Rabbani, U.S. ~~patent application Ser. No. 07/968,706, filed on Oct. 30, 1992, entitled "Capture Sandwich Hybridization Method and Composition," now allowed, that was published as European Patent Application Publication No. 0 159 719 A2 on Oct. 30, 1985. The contents of the foregoing U.S. patent application is incorporated herein by reference~~Patent No. 5,288,609, the contents of which are incorporated herein by reference .

Please amend the second paragraph on page 17 as follows:

It should not go unrecognized or even unappreciated that the foregoing nucleoside triphosphate and nucleoside triphosphate analogs can be unmodified or modified, the latter involving modifications to the sugar, phosphate or base moieties. For examples of such modifications, see Ward et al., U.S. Pat. No. 4,711,955; Engelhardt et al., U.S. Pat. No. 5,241,060; Stavrianopoulos, U.S. Pat. No. 4,707,440; and Wetmur, Quartin and Engelhardt, U.S. patent ~~application Ser. No. 07/499,938, filed on Mar. 26, 1990, the latter having been disclosed in European Patent Application Serial No. 0 450 370 A1, published on Oct. 9, 1991~~No. 5,958,681,~~. The contents of the foregoing U.S. patents and patent application are incorporated by their entirety into the present application~~ the contents of which are incorporated in their entirety.

Please amend the paragraph bridging pages 18 and 19 as follows:

Modifications, including chemical modifications, in the composition of the primers would provide for several novel variations of the invention. See, for example, U.S. Pat. Nos. 4,711,955; 5,241,060; 4,707,440; and ~~U.S. patent application serial no. 07/499,938~~U.S. Patent No. 5,958,681, *supra*. For example, substitution of the 3' hydroxyl group of the primer by an isoteric configuration of heteroatoms, e.g., a primary amine or a thiol group, would produce chemically cleavable linkers. In the case of thiol excess of another thiol in the reaction mixture will cleave the phosphorothioate linkers which is formed after the initiation of polymerization, thus allowing the DNA polymerase to reinitiate polymerization with the same primer. Thus, in this variation repeated syntheses can begin from a modified, hybridized primer providing a significant increase in the synthesis of DNA.

Please amend the section "In vivo Synthesis of Nucleic Acid" from pages 27-30 as follows:

In vivo Synthesis of Nucleic Acid

This invention describes a cassette or nucleic acid construct into which any nucleic acid sequence can be inserted and which can be used as a template for the production of more than one copy of the specific sequence. This cassette is a nucleic acid construct containing a sequence of interest, which within or present within, the cell produces nucleic acid product which is independent or only partially dependent on the host system. The cassette or nucleic acid construct may be characterized as a promoter-independent non-naturally occurring, and in one embodiment comprises double-stranded and single-stranded nucleic acid regions. This construct contains a region in which a portion of the opposite strands are not substantially complementary, e.g., a bubble (even comprising at least one polyT sequence), or loop, or the construct comprises at least one single-stranded region. The construct is composed of naturally occurring nucleotides or chemically modified nucleotides or a synthetic polymer in part or a combination thereof. These structures are designed to provide binding of polymerizing enzymes or primers and the modifications provide for nuclease resistance or facilitate uptake by the target cell.

Referring to the constructs (A-F) depicted in FIG. ~~3~~1, the single stranded regions described in the constructs will contain coding sequences for nucleic acid primers present in the cell to facilitate initiation points of DNA polymerase in said cell. In the case of RNA polymerase, these constructs constitute ~~promotor~~ promoter independent binding and initiation of RNA polymerase reaction. These constructs can be used *in vitro* and *in vivo* for production of nucleic acids. The position of the single stranded region adjacent to the double stranded specific sequence would provide a specific and consistent transcription of these specific sequences, both *in vitro* and *in vivo* independent of promoter. The replication (DNA) or transcription (RNA) products of these constructs can be single stranded nucleic acid which could have a sense or antisense function or could be double stranded nucleic acid.

In FIG. 1~~3 (A)~~A, a large bubble is located in the construct. In FIG. ~~13(B)~~1B, the two strands are noncomplementary at their ends, and thus do not form a bubble. In FIG.

~~13(C)~~1C, a double bubble is formed due to noncomplementarity at both ends. In FIG. ~~13(D)~~1D, a single-stranded region is shown in the middle of the construct leading to a partially single-stranded region (and no bubble formation). FIG. ~~13(E)~~1E depicts a bubble at one end of the construct (compare with the two bubbles in the construct shown in FIG. ~~13(C))~~1C. In FIG. ~~13(F)~~1F, a single bubble in the middle of the construct is shown. It should be readily appreciated by those skilled in this art that the above-depicted embodiments are representative embodiments not intended to be limiting, particularly in light of the present disclosure.

In vivo these constructs, with a specific primer present in the cell can initiate nucleic acid synthesis. When these primers are RNA, after initiation of nucleic acid synthesis, they can be removed by the action of ribonuclease H, thus vacating the primer binding sequence and allowing other primer molecules to bind and reinitiate synthesis. The cellular nucleic acid synthesizing enzymes can use these constructs to produce copies of a specific nucleic acid from the construct. Shown in FIG. ~~14~~2(A-F) are corresponding illustrations of the constructs in FIG. ~~13~~1(A-F), except that the production arrows (points and directions) are indicated.

These constructs could contain more than one specific nucleic acid sequence which in turn could produce more than one copy of each specific nucleic acid sequence. If two independent nucleic acid products are complementary, then they could hybridize and form multiple copies of a new double stranded construct that could have the properties of the novel construct. Furthermore they could contain ~~promotor~~ promoter sites such as the host ~~promotor~~ promoter therefore serving as an independent nucleic acid production source (the progeny).

Please amend the two paragraphs between page 35, line 4 to page 36, line 17 as follows:

In certain detection formats the primers may be removed from the reaction mixture by capturing the product through direct capture (Brakel et al., U.S. patent application Ser.

No. ~~07/998,660~~07/459,030, filed on ~~Dec. 23, 1992~~ Dec. 29, 1989, now abandoned, the contents of which have been disclosed in European Patent Application 0 435 150 A2, published on Jul. 3, 1991; and the contents of which are also incorporated by reference herein), or sandwich capture. (Engelhardt and Rabbani, allowed U.S. ~~patent application Ser. No. 07/968,706, *supra*~~ Patent No. 5,288,609), or by modifying the primers at the 3' end with biotin or imminobiotin without an arm or a very short arm such that the avidin will recognize only the unincorporated primers (single stranded form) but not the incorporated due to the double stranded form and the short length of the arm. Additionally, the primer may be labeled with ethidium or other intercalating moiety. In this condition, the ethidium or other intercalating moiety may be inactivated (Stavrianopoulos, U.S. ~~patent application Ser. No. 07/633,730, filed on Dec. 24, 1990~~Patent No. 5,989,809, ~~published as European Patent Application Publication No. 0 492 570 A1 on Jul. 1, 1992;~~ the contents of which are incorporated by reference) in the unhybridized oligo and not in the hybridized oligo:target.

Another aspect of this invention herein described is to provide for a conjugate of a nucleic acid polymerizing enzyme (RNA polymerase) with a nucleic acid construct said nucleic acid construct contains an initiation site such as a ~~promotor~~ promoter site for the corresponding RNA polymerase which is capable of producing nucleic acid both in vivo and in vitro. The enzyme could be linked directly to the nucleic acid or through a linkage group substantially not interfering with its function or the enzyme could be linked to the nucleic acid indirectly by a nucleic acid bridge or haptene receptor where the enzyme is biotinylated and the nucleic acid construct contains an avidin or *vice versa* or when the nucleic acid construct contains sequences for binding proteins such as a repressor and an enzyme linked to said nucleic acid binding protein (U.S. Pat. No. 5,241,060, *supra*, and Pergolizzi, Stavrianopoulos, Rabbani, Engelhardt, Kline and Olsiewski, U.S. patent application Ser. No. ~~08/032,769~~491,929, filed on ~~Mar. 16, 1993~~May 5, 1983, now abandoned and published as European Patent Application Publication No. 0 128 332 A1 on Dec. 19, 1984, the latter having been "allowed" by the European Patent Office, and further incorporated by reference herein).

Please amend Example 1 on page 43 as follows:

Primers

A set of twenty single stranded oligonucleotide primers (SEQ ID NOS:2-21), fifteen nucleotides long, were chemically synthesized.

The first set of 10 ~~primers~~ primers (SEQ ID NOS: 2-11) was complementary to one strand of M13mp18 replicative form (RF) starting at base 650 and extending to base 341. An interval of 15 nucleotides separated successive primers. The second set of 10 primers (SEQ ID NOS: 12-21) contained sequences identical to the single-stranded M13mp18 phage genome starting at base 351 and extending to base 635, again with 15 nucleotide gaps separating successive primers. There is a complementarity of 5 bases between opposing primers, but at an ionic concentration of 0.08M NaCl and 45ºC. these primers will not hybridize to each other. The sequences of the primers are shown in FIG. 6.

ARRANGEMENT OF OLIGONUCLEOTIDE PRIMERS IN AMPLIFICATION REACTION

1	2	3	4	5	6	7	8	9	10
20	19	18	17	16	15	14	13	12	11

Primer 1 (SEQ ID NO:2) begins at base 650 and primer 11(SEQ ID NO:12) begins at base 351.

Please amend Example 15 on page 61 as follows:

Example 15

T7 Promoter Oligonucleotide 50 Mer Labeled with Ethidium

An oligonucleotide 50-mer including the T7 promoter region of IBI 31 plasmid constructs (plasmid sequences derived from manufacturer, International Biotechnology, Inc.) was synthesized. Its sequence is as follows (SEQ ID NO:23):

3'-TAC T*AA T*GC GGT* CT*A T*AG T*T--AA TCA TGA AT--T AAT* TAT* GCT* GAG T*GA T*AT* C-5',

where T* represents allylamine dU, and therefore ethidium modification and the 10 base sequence set off by dashes (--AA TCA TGA AT-- of SEQ ID NO:23) was introduced to provide a restriction enzyme site.

Please amend the paragraph at page 62, lines 6-16 as follows:

The effect of this oligonucleotide on in vitro transcription by T7 and T3 polymerases from an IBI 31 plasmid construct (pIBI 31-BH5-2) and from a BlueScript II plasmid construct (pBSII/HCV) was studied. See FIG. 18 which contains the same target sequences, but in a "split" arrangement. The polylinker sequences of these plasmids are given in FIG. 18 (SEQ ID NOS:22-27). Comparing the effect of the oligo on these two different target template serves to partially control for the possible non-specific inhibitory effects of ethidium groups on the RNA polymerases because the oligonucleotide would be expected to inhibit transcription from any template containing an appropriate promoter regardless of the "split" if the effect were due to the oligo's interaction with the polymerase rather than with the template.